Achievement of Interlaboratory Uniformity - A Summary of Work Carried out by the EDNAP Group

P GILL[a], S WOODROFFE[a], W BÄR[b], B BRINKMANN[c], A CARRACEDO[d], B ERIKSEN[e], S JONES[f], A D KLOOSTERMAN[g], B LUDES[h], B MEVAG[i], V L PASCALI[j], M RUDLER[k], H SCHMITTER[l], P M SCHNEIDER[m] and J A THOMSON[n]

[a]Home Office Central Research and Support Establishment, Aldermaston (UK), [b]Institute of Legal Medicine, University of Zurich (Switzerland), [c]Institute of Legal Medicine, University of Münster (FRG), [d]Department of Legal Medicine, University de Santiago de Compostela (Spain), [e]Institute of Forensic Genetics, Copenhagen (Denmark), [f]Metropolitan Police Forensic Science Laboratory, London (UK), [g]Forensic Science Laboratory, Rijswik (The Netherlands), [h]Institute of Legal Medicine, University of Strasbourg (France), [i]Institute of Forensic Medicine, University of Oslo (Norway), [j]Institute of Legal Medicine, Catholic University, Rome (Italy), [k]Laboratoire de Police Scientifie de Paris, Ministere de L' Interier (France), [l]Bundeskriminalamt, Wiesbaden (FRG), [m]Institute of Legal Medicine, University of Mainz (FRG), [n]Department of Haematology, London Hospital Medical College (UK).

INTRODUCTION

This paper describes a collaborative exercise intended to demonstrate whether uniformity of DNA profiles results could be achieved between different European laboratories. It was shown that this goal would be obtained provided that a common protocol was followed (specifically the use of a common electrophoretic buffer is the most important parameter).

Generally lower molecular weight loci (with lower molecular weight fragments) such as YNH24 perform better than higher molecular weight loci such as MS43a. The results of the exercise are discussed in relation to the objectives of the European DNA profiling group (EDNAP).

THE WORK OF THE EDNAP GROUP

In 1989 the EDNAP group began a series of experiments to determine whether standardisation of DNA profiling systems was possible. The first series of experiments was reported by Schneider et al. (1991). Each laboratory analysed a series of samples using its own protocols (the only standardisation was use of HinfI as the restriction enzyme, and use of a common ladder marker). It was demonstrated that the sizes of fragments determined by different laboratories were within a match window of 10%. Although it was possible to compare directly results between laboratories using this wide window, interpretation and allocation of statistical significance was more difficult when such large differences were obtained between laboratories.

The second series of experiments described in this paper was carried out in order to determine whether comparable results could be obtained if different laboratories used the same protocol. Clearly, it was not possible to standardise completely because different equipment was in use. However, it was possible to standardise on electrophoretic buffer and make of agarose. Also each laboratory was supplied with a full protocol and samples of DNA; equipment was not standardised.

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A comparison of the within and between laboratory measurement error has been evaluated.

MATERIALS AND METHODS

DNA was bulk extracted by one laboratory (Central Research and Support Establishment CRSE) from 3 different blood samples. Half was then restricted by this laboratory with 30x excess HinfI before distribution and the other half was supplied to laboratories unrestricted so that the effect of different restriction methods could be compared. In addition, HinfI restricted K562 (genomic control; Promega) and Amersham lambda ladder was supplied to each laboratory. Each laboratory was supplied with a full protocol to follow. Probes YNH24 and MS43a were used.

RESULTS AND DISCUSSION

The differences between fragment sizes from each half of the gel were low (< 0.5%kB); it is very unlikely that different restriction buffers or different manufacturers HinfI restriction enzyme contribute to the effect.

Evett et al. (1990) introduced a method of analysis which enabled a large number of comparisons to be carried out from a relatively small database. The principle of the test involves comparison of each pair of band measurements with every other pair in the database referring to a match guideline. Part of the datafile of sample A (YNH24) is as follows:-

Identifier (laboratory)	Band Weights (bp) 1	2
1	4564	2724
2	4584	2735
3	4633	2776

If a match guideline is set at 2% then sample 1, band 1 and sample 2, band 1 would be deemed a match provided that each was within the range of ±1% of the mean band weight (xb) of the two fragments, ie. xb = (4564 + 4584)/2 and the match window is therefore xb ± (1/100)*xb. Of course it would also be a requirement for the second band in both samples to be within the guideline before a match was declared.

In this exercise datafiles were produced, (one for YNH24 and the other for MS43a). In each datafile there were up to 22 observations (from 11 laboratories). A match criterion was set between 2-8%. If a comparison of fragments from samples 1 and 2 was within the match criterion then a match was recorded. Sample 1 was then compared with sample 3 and so on to the end of the file. If every sample was compared in this way there was a total of n*(n-1)/2 comparisons, ie. up to 231 in this example. This process effectively simulates what would happen if a large number of casework samples were analysed in different laboratories and then compared with each other. The Home Office Forensic Science Service uses a match guideline of 2.8% for the reasons explained by Gill et al. (1991). Using this guideline

approximately 97.9% of samples probed with YNH24 match between gels and between 6 different UK laboratories all using the same protocol. Use of the European protocol produced results which were comparable to (or perhaps better) than those detailed by Evett et al, (1989) and Gill et al, (1991) because a 2.8% guideline resulted in >99% matches for all YNH24 samples tested. In general, use of a 2.8% window with MS43a data was not as definitive. This was to be expected because the variation in measurement error increased with molecular weight (Gill et al, 1990).

This was a useful but simple exercise which laboratories wishing to compare results can follow. It requires only basic computing expertise to write the necessary program.

So far only the quality of the match has been considered in this paper (ie. the numerator of a Bayesian likelihood ratio). Evett et al, (1991) has examined the combined effects of the numerator, denominator (ie. band frequency) and correlation coefficient and concludes that with YNH24 and MS43a using the current EDNAP protocol, between laboratory comparisons of the same DNA sample would result in a likelihood ratio >100, with 50% of matches resulting in a likelihood ratio $>10^5$.

CONCLUSION

Once a protocol has been established, and agreed, then laboratories need a method to monitor the results which they achieve using an agreed quality control system which incorporates universal ladder markers and genomic controls for sizing purposes. This will enable confident interpretation between laboratories, since each autoradiograph effectively contains a universal control which can be independently checked. It would be necessary for the size of the control to fall within designated limits before inclusion into a database. Provided these simple rules are followed there would be no reason why laboratories could not exchange information and combine databases, although the latter would be dependent upon considerations relating to population structure.

The achievement of uniformity and quality control methods are currently the subject of active discussion within EDNAP and within the international DNA commission of the Forensic Haemogenetics Society.

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